IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Roy R. Teller
Art Unit	:	1654
Applicant	:	William S. Dalton, Jason S. Damiano, Anne E. Cress
Serial No.	:	10/024,017
Filed	:	December 21, 2001
For	:	Compounds and Methods For Modulating Cell-Adhesion Mediated Drug Resistance

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF WILLIAM S. DALTON, Ph.D., UNDER 37 C.F.R. § 1.132

Sir:

I, William S. Dalton, Ph.D., of the University of South Florida, hereby declare:

THAT, my *curriculum vitae* is attached hereto as Exhibit A;

THAT, I am a named inventor on the above-referenced patent application;

THAT, I have read and understood the specification and claims of the subject application and the Office Action dated June 17, 2003;

AND, being thus duly qualified, do further declare:

1. In the above-referenced Office Action, the Examiner indicates that the prior art teaches that there is unpredictability regarding *in vivo* therapy and persons skilled in the art would not associate the *in vitro* results described in the patent application with *in vivo* therapeutic efficacy. The Examiner cites page 2, lines 20-21, of the patent application, and the Dermer publication (*Biotechnology*, 12:320, 1994), in support of this assertion.

2. With regard to page 2, lines 20-21, of the patent application, the Examiner is taking the statement out of its proper context, resulting in an overly broad generalization. Specifically, page 2 of the application states "It is also known that certain resistanc mechanisms may only be functional

in vivo, where tumor cells continue to interact with environmental factors such as extracellular matrix (ECM) and cellular counter-receptors" (emphasis added). The examples in the patent application include cytotoxicity assays using, for example, human drug-sensitive myeloma cells (8226/S cells) and human drug-resistant myeloma cells (8226/LR5, L-phenylalanine-mustard-resistant cells; and 8226/DOX6, doxorubicin-resistant cells) that are adhered to fibronectin, a major component of ECM. As indicated at page 12, lines 3-6, and page 17, lines 6-10, of the patent application, drug-sensitive cells of the human myeloma 8226 cell line express both VLA-4 ($\alpha_4\beta_1$) and VLA-5 ($\alpha_4\beta_1$) integrin fibronectin receptors, and the cells adhere to fibronectin through β1 integrin reactions. Thus, the *in vitro* conditions prepared in the cytotoxicity assays described in the application do mimic or approximate the *in vivo* conditions most relevant for determination of cell adhesion-mediated drug resistance. It is acknowledged that the environmental context into which cells of tumor cell lines are placed has important consequences, particularly for the multi-drug resistant phenotype. However, these factors were appreciated and factored into the design of the experiments described in this patent application, the results of which are thus predictive of *in vivo* activity.

3. The Dermer publication, which asserts that tumor cell lines do not mimic conditions in the human, is an editorial-style article as demonstrated by both the journal's disclaimer at the left of the page and the statements contained therein. For example, Dermer asserts that data from tumor cell lines 'cannot be relevant to cancer initiation in humans.' The enormous amount of research that continues to be carried out using tumor cell lines as cancer models contradicts Dermer's decade-old statement. Moreover, using statistical analysis and a survey of the clinical and pre-clinical literature, Voskoglou-Nomikos *et al.* (Voskoglou-Nomikos T. *et al.*, *Clin. Cancer Res.*, 9(11):4227-4239, 2003) recently verified that the *in vitro* human cell line model was predictive of Phase II clinical trial performance of cancer drugs. The relevance of the Dermer publication is also questionable because of what is not stated. For example, Dermer does not comment on the vast array of experimental conditions that can be applied to cells of tumor cell lines to approximate the *in vivo*

microenvironment, nor does Dermer address the utility of tumor cell lines to evaluate potential modulators of the multi-drug resistance phenotype.

4. There is a reasonable correlation between *in vitro* results obtained using multi-drug resistant tumor cell lines and modulation of drug resistance *in vivo*, and this correlation is recognized in the scientific literature. Salmon *et al.* (Salmon S. *et al.*, *Blood*, 78(1):44-50, 1991) evaluated verapamil as a chemosensitizer for reversing multi-drug resistance in multiple myeloma both *in vitro* and in clinical trials. Verapamil was capable of sensitizing myeloma cells that exhibited resistance to doxorubicin and vincristine *in vitro*, and reversing multi-drug resistance in some patients with VAD-refractory myeloma. Bellamy *et al.* (I) (Bellamy W., *et al.*, *Am. J. Pathol.*, 142(3):1993) have established an *in vivo* model of human multiple myeloma in the severe combined immunodeficient (SCID) mouse using both the RMI 8226 human myeloma cell line and P-glycoprotein-expressing multidrug-resistant 8226/C1N subline. Doxorubicin was effective in treating the drug-sensitive 8226 human-SCID xenografts. This study shows that the *in vitro* drug resistance phenotype was also observed in the *in vivo* model, indicating that the drug resistance phenotype characterized *in vitro* is preserved when the tumor cells are grown within an animal. Peptides exhibiting *in vitro* multi-drug resistance modulating activity can be screened for similar activity using this or other animal models of multi-drug resistance without resort to undue experimentation. Bellamy *et al.* (II) (Bellamy W. *et al.*, *Adv. Clin. Chem.*, 31:1-61, 1994) indicate that modulators of multi-drug resistance, such as verapamil, quinidine, and cyclosporine A and its analogs, retain their activity in animal models. In the *in vivo* setting, some compounds have been shown to increase survival time by 40% to 200%, compared to control animals (see page 34, lines 30-35, of Bellamy *et al.* (II)).

5. To further support the conclusion that our invention is enabled by the teachings of our specification, submitted herewith as Exhibit B is data demonstrating that adhesion of patient myeloma cells to fibronectin does confer resistance to melphalan. These data show that the CAM-DR phenotype established with our *in vitro* cell lines is indeed operative in primary myeloma patient specimens, consistent with our teachings.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

Further declarant sayeth naught.

Signed: _____
William S. Dalton, Ph.D.

Date: October 17, 2003